Enzyme Kinetics

Introduction

We're going to do a whirlwind through thermodynamics and enzyme kinetics. While there are a few equations, the concepts are far easier than you might think, AND there's not much calculating.

Thermodynamics: ΔG

Thermodynamics answers two questions: will the reaction go, and how fast will it do so.

ΔG answers, **will the reaction occur**?

If the **ΔG is > 0** (positive), then it **won't go spontaneously**. Energy is required by the system to go—energy has to be added in order for it to happen.

If the **ΔG is 0**, that is neither positive nor negative; the two states are in **equilibrium**. The substrate (what you start with) will go to product (what you end up with) and vice versa.

If the **ΔG is < 0** (negative), then the reaction **will occur spontaneously**. Energy is derived from the reaction and it's often **an irreversible reaction**—going the other way back to substrate would require energy to be put in.

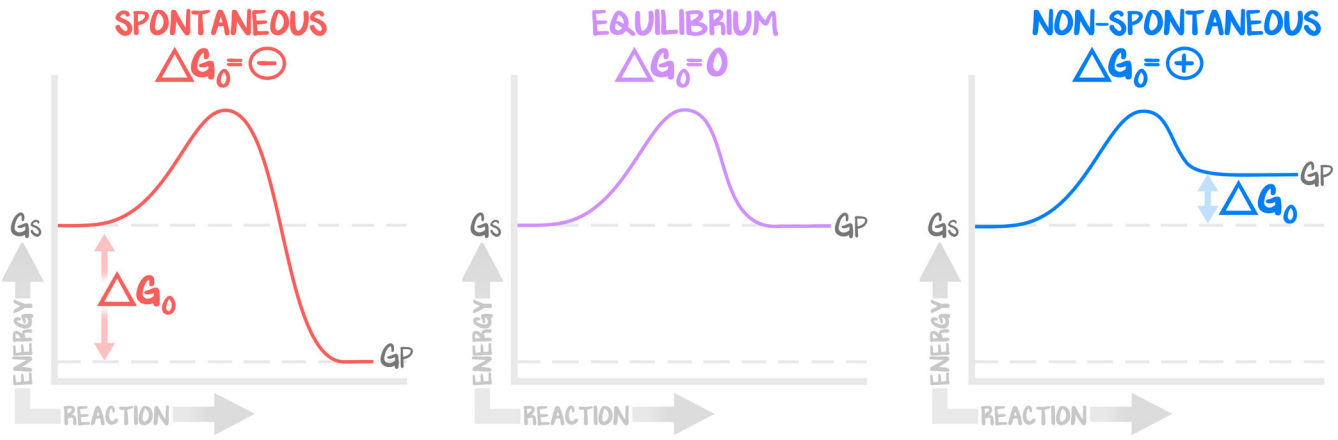

Figure 17.1: ΔG
The different possibilities for the ΔG of the reaction. If the products have less energy than the substrate, the ΔG is negative, and the reaction is spontaneous. If the products have more energy than the substrate, the ΔG is positive, and the reaction is non-spontaneous. If the products and substrate have equal energy, the ΔG is 0, and they are said to be in equilibrium.

ΔG is the change of energy from what you start with to what the product is. It is. It can't be changed, adjusted, or shifted. It's true of the reaction.

We show graphically what we said in words. ΔG is calculated by subtracting the starting energy (**Gs**) from the product energy (**Gp**). If ΔG is negative, then it's a **downhill reaction** and will **go spontaneously**. If ΔG is positive, then it's an **uphill reaction**.

Thermodynamics: Velocity

V (rate) answers, **will the reaction occur quickly?** The **rate** can be influenced by enzymes. V is determined by ΔG‡. ΔG was the Product - Substrate. ΔG‡ is the **activation energy**. Even though they both have a "delta" and a "G," ΔG and ΔG‡ are completely separate values representing completely different things.

While the net result of a reaction may have a negative ΔG (a spontaneous reaction), if the activation energy is quite high the reaction will go slowly. The **higher ΔG‡**, the **higher the activation energy**, the **slower the reaction**, and therefore **the lower V** is.

See activation energy as the "hump" or the "wall" that has to be hurdled. Once that critical threshold is gotten over, nothing can stop the reaction from happening. What **enzymes do** is **catalyze** the reaction, **reducing the activation energy**, lowering the wall, the hump, the barrier. Hence, enzymes increase the rate of reaction.

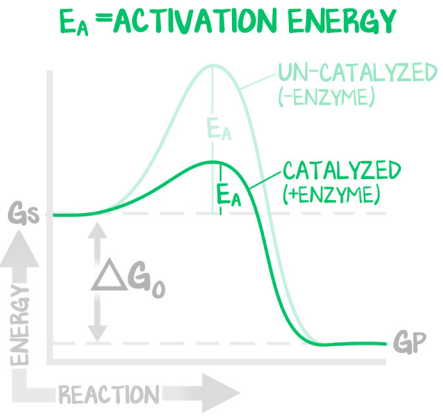

Figure 17.2: Activation Energy, EA Dagger
Enzymes can affect ONLY VELOCITY by reducing E_A (the activation energy) and cannot influence the ΔG of the reaction.

Kinetics (Michaelis-Menten)

Enzyme kinetics comes down to two things: K_M and V_{max}.

V_{max} is the maximum rate possible given the amount of enzyme. The idea is that enzymes can become **saturated**. At a certain point, binding sites are taken up; any one enzyme can only work so hard. At V_{max}, adding **more substrate will NOT increase the rate**. The only way to increase V_{max} is by **adding enzyme**, thereby taking a saturated system and un-saturating it. V_{max} can be increased within the context of gene regulation by increasing production of a given enzyme—more enzyme, higher V_{max}.

K_M is a marker of **enzyme affinity**. It's determined by taking ½ the V_{max} on the y-axis, moving to the right until the curve is encountered, and then identifying what subtrate concentration this is. A **high-affinity** enzyme will require almost no substrate to start working, and therefore will have a **low K_M**. A **low-affinity** enzyme will require a lot of substrate to get going, and therefore will have a **high K_M**.

See the correlates between thermodynamics and kinetics.

In thermodynamics, ΔG is inherent to a reaction. It doesn't change. What enzymes do is change the rate (by adjusting activation energy).

K_M is inherent to an enzyme. It doesn't change. Just like ΔG doesn't change. K_M is used to compare two enzymes to each other. Enzyme quantity (concentration) can increase the maximum rate (V_{max}), but K_M can't be changed.

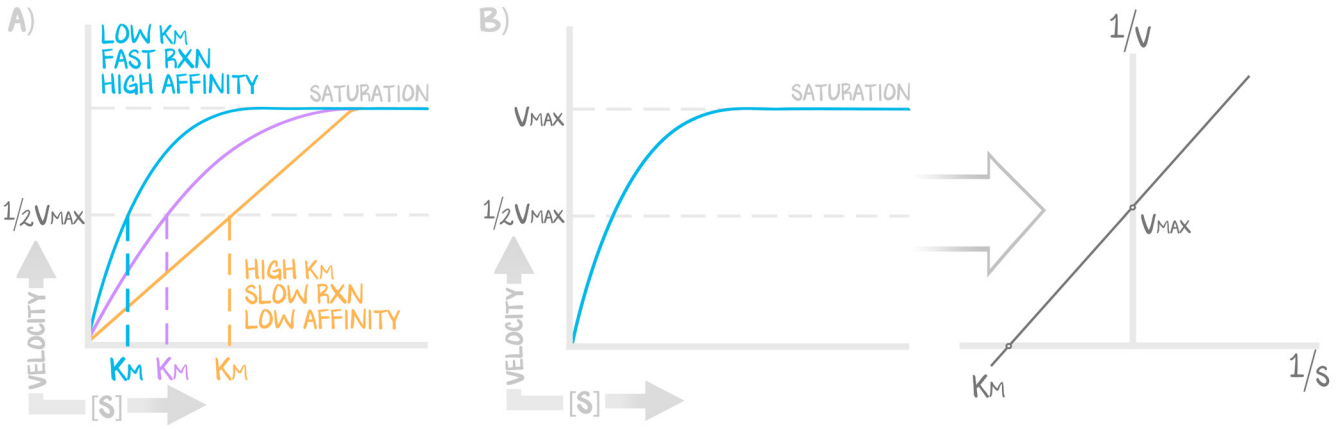

Figure 17.3: Enzyme Kinetics
(a) Demonstrating different enzymes showing difference in velocity at a given concentration (affinity, K_M). (b) Michaelis-Menten kinetics demonstrating a logarithmic curve, which could mathematically be turned into a more predictable straight line with a slope by inverting the x and y axes in the Lineweaver-Burk graph.

Lineweaver-Burk

This takes what we've learned about kinetics and gets you ready for test prep, learning how to interpret these double-reciprocal plots . . . or just memorizing which is which.

Lineweaver-Burk curves are the Michaelis-Menten equation plotted to be a **straight line**, such that any two points yield the entire predictability of the enzyme—both K_M and V_{max}. This is where things get math-side-down'd. Be careful not to miss a step.

The y-axis remains a measurement of rate, but it's the reciprocal—that is 1/**v**. As you get higher on the y-axis, the higher 1/v gets, and the **slower the reaction**. It's that 1/v that can cause confusion. Again, as you go higher on the y-axis, the value of 1/v gets larger, meaning the actual rate is going down. The rate goes DOWN as you go UP the y-axis. The **y-intercept** is therefore $\mathbf{1/V_{max}}$.

The x-axis remains a measurement of concentration, but it's the reciprocal, 1/[S]. The **x-intercept** remains the K_M portion, only expressed as $\mathbf{-1/K_M}$. The farther to the left you get, as the numbers get larger in magnitude, the smaller $\boldsymbol{K_M}$ will be, and the **better affinity it will have**. We didn't do math on purpose. If you **move left**, $\boldsymbol{K_M}$ **goes down**, and therefore, **affinity goes up**. If you move right, K_M goes up, affinity goes down.

Ready to see how easy this is? Y-axis is V_{max}. Go up the y-axis, get a lower V_{max} (it's inverted). X-axis is K_M. Go left, get a lower K_M, and higher affinity (more negative means higher affinity). The y-axis is one step; go up, V_{max} goes down. The x-axis is two steps, but the point is that left is better affinity.

One more time.
UP ON THE Y-AXIS = DOWN V_{MAX} = SLOWER
LEFT ON THE X-AXIS = DOWN K_M = HIGHER AFFINITY

We'll use this more in the next lesson.